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ITS REDUCTION PRODUCTS, THE KERATEINES

By JOSEPH ISAAC ROUTH

A DISSERTATION SUBMITTED IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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THE ENZYMATIC DIGESTION OF WOOL*

BY JOSEPH I. ROUTH AND HOWARD B. LEWIS

(From the Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor)

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It has generally been accepted that keratins are not attacked by enzymes. In a review of the literature by Barritt (1) the property of non-digestibility was an essential part of the definition of a keratin. However, there has been evidence for the digestion of wool by pancreatin. Wool that had been suspended in solutions of varying pH (2 to 10) for 48 hours at room temperature was slowly attacked by the enzymes (2). Wool previously treated at pH 10 was most readily digested. If the alkali was stronger (pH 11) and the temperature was raised to 37°, the same period of treatment produced a keratin that was extensively attacked by pancreatin over long periods of time (200 to 400 hours) (3). Keratins have been found to be digested by the crop juice of predatory birds (4) and by the intestinal juice of the larvæ of a species of clothes-moth (5). Several investigators have observed enzymatic digestion of protein derivatives prepared by the action of oxidizing and reducing agents on wool and hair (5-8).

The present investigation is concerned with the enzymatic hydrolysis of wool keratin and of its derivatives produced by the action of the reducing agent, thioglycolic acid. For comparison, a well characterized protein, casein, was studied under similar conditions.

EXPERIMENTAL

The rate of digestion of the proteins by trypsin was followed by the determination in the digest of the nitrogen not precipitable by

* The essential portions of this paper were presented before the Thirty-second meeting of the American Society of Biological Chemists at Baltimore, March 30 to April 2, 1938.

20 per cent trichloroacetic acid.¹ Substrates of approximately 5 per cent concentration were made by suspending the various keratin preparations in sodium hydroxide solution (pH 8 to 9) to which were added 5 cc. of a 5 per cent solution of trypsin (Difco 1:110) per 100 cc. of suspension. The digests were covered with toluene and maintained at 35–40°. At intervals, 5 cc. samples were withdrawn and the protein material precipitated with an excess of the precipitant. After filtration, the nitrogen in the filtrates was determined by the Kjeldahl method.

The method of formol titration was also applied to the filtrates from the tryptic digests described above. In many cases, 5 cc. samples were boiled to inactivate the enzyme and analyzed by the formol titration method in the usual manner. The mixture after titration was immediately treated with the precipitating reagent and the non-precipitable nitrogen was determined in the filtrate. In this way, comparative determinations by the two methods could be made with the same aliquot of the protein suspension under identical experimental conditions.

The proteins were also subjected similarly to the action of pepsin. Substrates of approximately 5 per cent concentration were made by suspending the various proteins in 0.1 N hydrochloric acid to which were added 5 cc. of a 5 per cent solution of pepsin (Difco 1:20,000) per 100 cc. of suspension. Non-precipitable nitrogen was determined on aliquots as already described. In every case blanks were run on protein suspensions in which the enzyme had been inactivated by boiling.

An entire fleece of wool was obtained to insure an adequate supply of uniform starting material for the investigation. The fleece was first washed with gasoline and then with Ivory soap and lukewarm water. To insure complete removal of any fatty material the wool was extracted with warm alcohol and finally with warm chloroform. The cleaning process as outlined above was employed to prevent any alteration of the sulfur or cystine content of the wool. The average composition of the resulting keratin was 16.04 per cent nitrogen, 4.03 per cent sulfur, 12.90 per cent cystine, and 0.42 per cent ash. Nitrogen was determined by the Kjeldahl method and cystine, by the Rossouw-Wilken-

¹ Experiments that gave substantially the same results were also carried out with 4 per cent sulfosalicylic acid as a precipitating reagent.

Jorden modification of the Sullivan method (9). Sulfur was determined by the Benedict-Denis method after a preliminary treatment with nitric acid as outlined by Wilson and Lewis (10).

The untreated wool as prepared above appeared to be very slowly attacked by trypsin (Fig. 1). The digestion had increased to 5 to 7 per cent at the end of 30 days. It was thought that an increase in the state of subdivision of the wool fibers might result in a more rapid digestion by enzymes. 100 gm. of untreated wool were ground for approximately 75 hours in a ball mill and the resulting product, Powdered Wool A, which contained 20.8 per

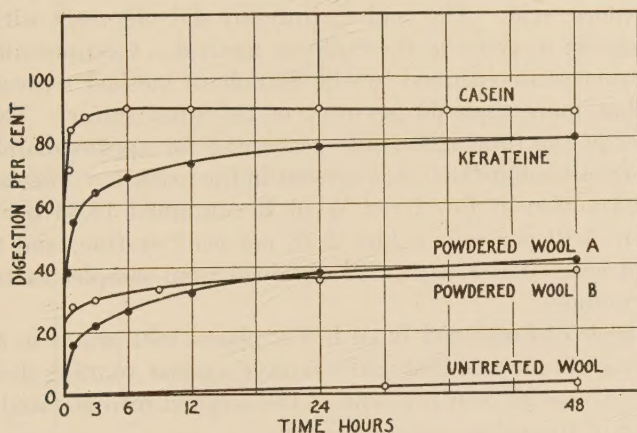


FIG. 1. The tryptic digestion of wool proteins and casein as measured by increases in the non-precipitable nitrogen fraction of the digests. The curves are corrected for blank determinations on similar digests with boiled enzyme solutions.

cent ash, had an average composition of 15.80 per cent nitrogen, 4.03 per cent sulfur, and 10.40 per cent cystine calculated on an ash- and moisture-free basis. Another 75 gm. sample of wool was ground for approximately 125 hours. This material, Powdered Wool B, contained a notably higher content of ash, 48.23 per cent, 15.50 per cent nitrogen, 4.24 per cent sulfur, and 8.74 per cent cystine.

In preliminary experiments it was observed that an appreciable amount of nitrogen was dissolved from the powdered wool samples immediately after the suspensions were prepared. 6.94 per cent

of the total nitrogen and 6.85 per cent of the total sulfur of Powdered Wool A were extracted by water. The contents of water-soluble nitrogen and sulfur of Powdered Wool B were 22.54 and 22.35 per cent of the total respectively. The water extracts of the powdered wools gave a biuret test, a slight precipitate with trichloroacetic acid, and positive Folin-Marenzi and cyanide-nitroprusside tests but produced no color with the Sullivan reagents. The extract from Powdered Wool B was analyzed for its cystine content by the Shinohara method (11). The color developed slowly and at the end of 24 hours was essentially of the same intensity as that developed after hydrolysis of the extract with hydrochloric acid. The acid hydrolysate did not react with the chromogenic reagents in the Sullivan method. Cystine sulfur in the extract as determined by the Shinohara method represented somewhat more than 60 per cent of the total sulfur. The remainder of the total sulfur was accounted for approximately by the inorganic sulfate that was present in the extract. The residue after extraction of Powdered Wool B contained 15.63 per cent nitrogen, 4.19 per cent sulfur, 9.78 per cent cystine, and 56.15 per cent ash. These data were obtained from samples extracted for 10 minutes.

A sample of Powdered Wool B was placed with water in a collodion membrane and dialyzed 3.5 days against running distilled water. Losses of 36.6 per cent of the original nitrogen and 34.1 per cent of the sulfur were observed.

The enzymatic hydrolysis of the powdered wool was investigated and a definite increase in digestibility over untreated wool was noticed (Fig. 1). Tryptic digests of the water extract from Powdered Wool B exhibited no appreciable increases in formol titration over a control digest. The residue, after the water extraction, was attacked at approximately the same rate as the original powdered wool.

Goddard and Michaelis (8) reported that the kerateines prepared from wool by the reducing action of alkaline thioglycolate solution were digested by trypsin and pepsin. No details of their experiments were given. A series of kerateines was prepared for enzymatic investigations. The composition of a typical kerateine was 15.60 per cent nitrogen, 4.82 per cent sulfur, 15.53 per

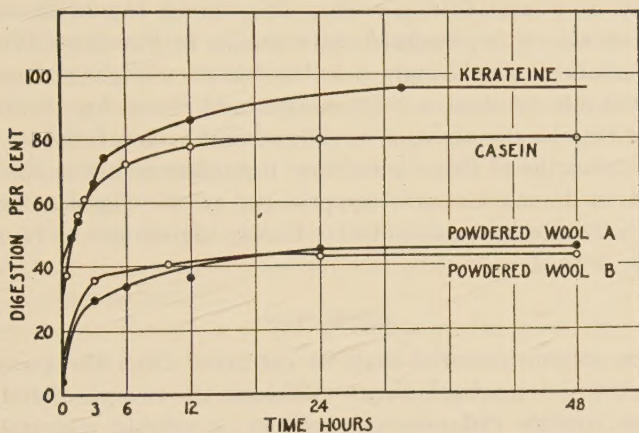


FIG. 2. The peptic digestion of wool proteins and casein as measured by increases in the non-precipitable nitrogen fraction of the digests. The curves are corrected for blank determinations on similar digests with boiled enzyme solutions.

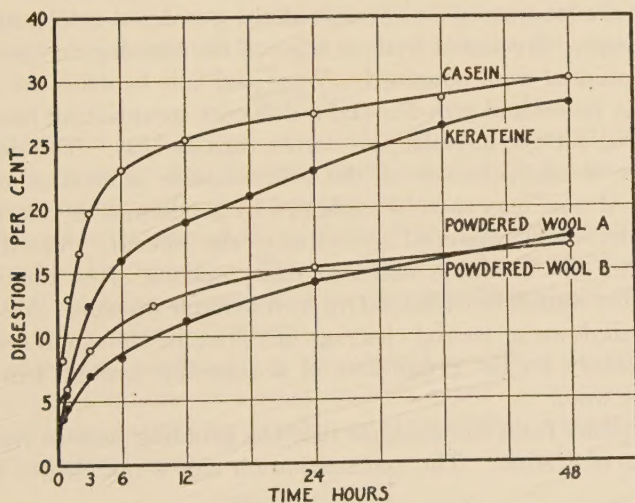


FIG. 3. The formol titration of tryptic digests of wool proteins and casein. The values represent percentages of the total nitrogen present as amino nitrogen as determined by the formol titration.

cent cystine,² and 0.15 per cent ash. Since the kerateines as prepared were in a powdered state similar to Powdered Wools A and B above, it was thought possible that they might also contain water-soluble substances. Extraction with water for 10 minutes did not remove any nitrogen or sulfur from the kerateines.

The behavior of these kerateines toward enzymes is shown in Figs. 1 to 3. As a control tryptic and peptic digestion experiments with casein were studied. Casein digests are to be found in Figs. 1 to 3.

DISCUSSION

Water-soluble material may be extracted from the powdered wool after prolonged grinding. Although the two powdered wool samples contain different proportions of soluble material, the extent of their digestion with trypsin and pepsin is practically identical. Since the water-soluble material was not attacked by trypsin, it appears that the action of the hydrolytic enzymes was limited to the water-insoluble fraction of the powdered wool.

Recently Professor W. T. Astbury of the University of Leeds visited the laboratory and examined the powdered wools under a microscope. He stated that no trace of the histological structure of the original wool remained. Wool and hair keratins are probably not individual proteins and a different composition has been suggested for the medulla and cortex regions (12). The demonstration of the presence of the water-soluble substance in the powdered wool may then be explained by mechanical action which made the soluble material accessible to the solvent. The digestibility of the insoluble material that remains after the water extraction cannot be explained by such a simple process. Mechanical breakdown or partial cleavage of the molecule would seem to be necessary for the production of a digestible protein from the original wool.

It appears from the analysis that the grinding process resulted in a loss of cystine. The explanation for this is not clear. How-

² The higher cystine value of the kerateine as compared with the original wool may be due to the presence of some cystine in the kerateine which has not been reoxidized. Since cystine in the Sullivan method is not reduced quantitatively to cysteine, the presence of the latter in a determination of total cystine would tend to yield somewhat higher values.

ever, the prolonged grinding may have effected a change in the cystine molecule to give a product no longer reactive in the color tests employed. The oxidation of a part of the cystine may explain the appearance of inorganic sulfates in the water extract of the powdered wool. Extraction of the original wool with weak hydrochloric acid as suggested by Marston (13) failed to alter its total sulfur content or to produce any inorganic sulfates in the extract. The results indicate that the inorganic sulfates in the water extract of powdered wool are not preformed and are probably an oxidation product.

In comparing the effect of the enzymes on the powdered wool samples with the effect on the kerateines and casein, it can be seen that the digestion of the latter was more rapid and extensive. While the rate of tryptic digestion of casein was slightly greater than that of the kerateines, the opposite was true with pepsin. In general, however, the behavior of the kerateines and casein toward enzymes was similar. Small amounts of the reducing agent thioglycolic acid, used in the preparation of the kerateines, when added to casein digests did not inhibit the action of the enzymes. Any differences in digestibility of the two proteins may be considered to be due to changes in the molecular structure.

SUMMARY

1. After wool was ground in a ball mill for prolonged periods of time, a water-soluble fraction containing nitrogen and sulfur could be extracted from the powdered wool.

2. The material extracted by water from powdered wool was not attacked by trypsin. The powdered wool and the residual material after water extraction of the powdered wool were readily attacked by trypsin and pepsin.

3. Kerateines produced by the reducing action of alkaline thioglycolate solutions on wool were hydrolyzed more extensively and much more rapidly by pepsin and trypsin than were the proteins of powdered wool.

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